The Fungus Beauveria tenella

Abstract. *Beauveria tenella,* an insect pathogen, grows well and sporulates freely in submerged culture. Enzymes that loosened bovine hair that were found in broth cultures were not produced in the presence of chitin, and substitution of peptone broth for peptone-glucose broth did not increase their concentration. Under certain conditions, oxalic acid was the main metabolic product in the peptone medium.

A culture of *Beauveria tenella* (Delacr.) Seim. (*1*) was isolated from laboratory air during a search for depilatory enzymes with unique properties. This organism produces enzymes which loosen the hair on animal hides, but the depilatory action was not particularly strong and did not differ, as far as we could tell, from that of enzymes produced by other fungi. In attempts to increase the production of depilatory enzymes various culture conditions were employed, and some interesting observations were made. MacLeod (*2*) has made an excellent study of the group of insect pathogens to which this fungus belongs.

Figure 1 shows the fruiting habit of the fungus when grown on an agar medium. Growth is very good in shake flasks on common media such as glucose-peptone-mineral salts solution. However, yeast extract and corn steep liquor increase the growth rate. The *p*H of such media decreases with growth and may fall to about 3.5 unless the solution is buffered. The optimum temperature for growth is about 28° to 30°C; growth was noticeably slower at 25°C, and there was no growth at 40°C. Conidia are readily produced in submerged culture. If calcium carbonate is added to the medium after 1 or 2 days' growth in a shake flask, the production of conidia becomes profuse. Newly formed and germinating conidia are often present in the same culture (Fig 1). This might be a useful method of producing spores for insect pathogenicity studies and control studies.

The hair-loosening action was strongest when the culture was grown at a *p*H above about 5.0. At a *p*H below this the activity was much decreased, and if the *p*H dropped below about 4.0, no hair-loosening activity was present.

This organism was induced to grow fairly well on chitin by gradually increasing the chitin content of the medium while decreasing the other nutrients (Fig. 1). However, no hair-loosening activity was produced when the organism was grown on this medium. Attempts to make the fungus attack keratin (horn and hoof meal) were not successful.

When the organism was grown on a peptone-meat extract medium (nutrient broth, Difco), the *p*H dropped from 6.8 to 3.7. This occurred repeatedly when 350-ml volumes of the inoculated broth [24 g/liter (3 times the usual strength)] were placed in Fernback flasks and shaken at 84 cy/min at a temperature of 28°C on a reciprocating shaker. However, if only 200-ml volumes of broth were used, a surprising result was obtained—the *p*H, instead of dropping, increased to 8.2 to 8.8. This was probably due to changes in the degree of aeration caused by the differences in volume.

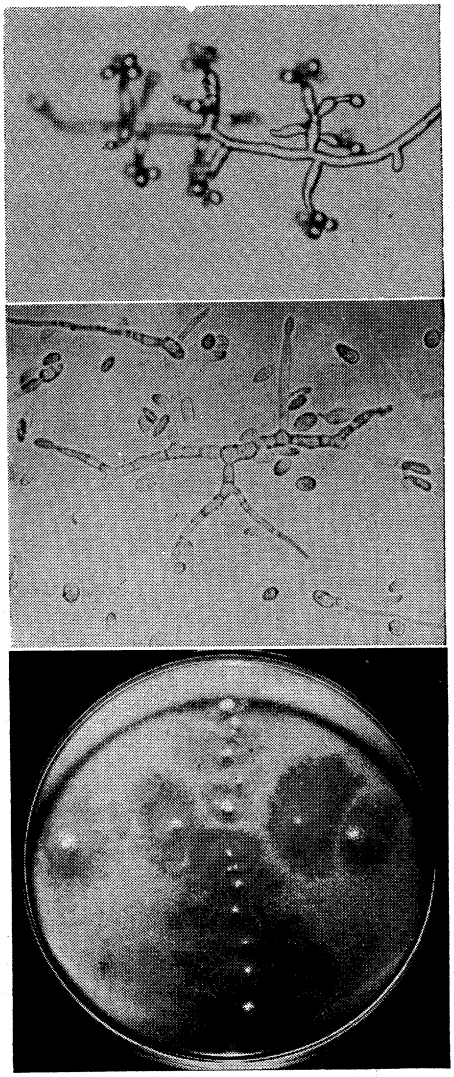

Fig. 1. *Beauveria tenella.* (Top) Conidiophores and conidia formed an agar medium (about × 760). (Middle) Concurrent spore germination and fruiting in submerged culture (about × 343). (Bottom) Chitin decomposition in an agar medium in a petri dish. The fuzzy white dots are mycelia with conidia and the dark areas are regions where the chitin has been digested (about × 0.59).

To identify the constituents responsible for the drop in *p*H, the fungus was grown on 350 ml of a peptone-meat extract medium, and a portion of this broth was treated with enough alcohol to make the solution 80 percent in alcohol. When this had stood 1 or 2 days a large amount of material (about 0.5 g) crystallized out. After filtration, the solution was examined by ion exchange and paper chromatography and found to contain oxalate as its main anion. Paper chromatography of the crystals and treatment with alkali showed them to be ammonium oxalate. The oxalate from the crystals and the oxalate remaining in solution were both precipitated as calcium salt and characterized by x-ray diffraction powder patterns. Approximately 20 percent of the original solids content of the broth was found to be oxalic acid. Peptone-meat extract broths in which the *p*H increased were not analyzed. A paper chromatogram of a broth from a culture grown on medium containing glucose showed no oxalic acid.

The production of oxalic acid from peptone by fungi has been discussed and documented by Foster (*3*), but as far as we know it has not been previously reported for fungi of this group.

The formation of unidentified crystals in the blood of insects infected by *Beauveria* species has been reported by Steinhaus (*4*). There is a very good chance that these crystals are the same as those we have isolated—ammonium oxalate.

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References and Notes

1. We thank Dorothy I. Fennell of the Mycology Laboratory, Pioneering Research Division, Quartermaster Research and Engineering Center, Natick, Mass., for making the identification.
2. D. M. MacLeod, *Can. J. Botany* **32**, 818 (1954).
3. J. W. Foster, *Chemical Activities of Fungi* (Academic Press, New York, 1949), p. 346.
4. E. A. Steinhaus, *Principles of Insect Pathology* (McGraw-Hill, New York, 1949), p. 374.

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